

## ONTOGENY OF DEFENSE AND ADAPTIVE COLORATION IN LARVAE OF THE COMMA BUTTERFLY, *POLYGONIA C-ALBUM* (NYMPHALIDAE)

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**ABSTRACT.** In many butterfly species early and late larval instars differ in coloration. The first three larval instars in the comma butterfly, *Polygonia c-album*, have coloration that, to the human eye, appears to be disruptively cryptic. The last two instars, which are defended by strong branching spines, are instead strikingly colored in white, black, and orange. It has been suggested that this coloration is also cryptic, mimicking bird droppings. We test the idea that this coloration is instead (or simultaneously) aposematic, using young chickens as models of bird predators. Some chicks readily ate third-instar larvae, both initially and after having tasted them, suggesting that no chemical defense is present in comma larvae. The chicks also ate dead fifth-instar larvae from which the spines had been removed. Chicks initially attacked fifth-instar larvae with intact spines, but learned to avoid them already after the first attack. This suggests an aposematic function of the coloration, which can have evolved by individual selection in this and many other nymphalid species with spiny larvae, because larvae were not harmed during the learning process. The evolutionary causes of the ontogenetic shift in defense tactics are discussed.

**Additional key words:** aposematism, mimicry, life history, predation, adaptive function.

Adaptive coloration in animals has been a very active research field in evolutionary biology over the years (e.g., Poulton 1890, Cott 1940, Kettlewell 1973, Sillén-Tullberg 1988, Malcolm 1990), and one in which the Lepidoptera have always featured prominently as model species. Adaptive coloration includes crypsis (helping to avoid detection by other animals), signalling, and thermoregulation. Crypsis can be achieved by having colors similar to the background, or by having mosaic patterns of spots or stripes that break up the contour and surface area of the animal (called “disruptive crypsis”; Cott 1940, Edmunds 1990). Signals can be of several types, including “aposematism,” defined as conspicuous colors advertising unprofitability to predators (Guilford 1990a, b). A phenomenon that may include aspects of both signalling and crypsis is “mimicry,” a term used for organisms that adaptively resemble another species, as in the cases of Müllerian and Batesian mimicry (Malcolm 1990). In the former case, unpalatable aposematic organisms mimic each other. In the latter case, a palatable organism mimics an aposematic one, capitalizing on its defenses. Sometimes the term mimicry is extended to cases when organisms mimic the shape and colors of objects or immobile organisms for the purpose of crypsis, for example as in stick insects (Edmunds 1990). Finally, variation in coloration can be due to different requirements for thermoregulation rather than predator avoidance (e.g., Shapiro 1976, Kingsolver & Watt 1983). For all of these types of (presumably) adaptive coloration, it is probably fair to say that the function is often assumed, but seldom tested.

The coloration of the first three larval instars in the comma butterfly, *Polygonia c-album* L. (Nymphalidae), appears to the human eye to be disruptively cryptic (Fig. 1A), at least when seen against a naturally variegated background of light and shadow. The last two instars, which are defended by strong branching spines, are much more strikingly colored in white, black and orange (Fig. 1B). The same type of coloration is present in the probable sister species to *P. c-album*, the Nearctic *P. faunus* (Scudder 1889, Scott 1986). It has been suggested that this type of coloration is also cryptic, the large continuous white area supposedly mimicking bird droppings (Thomas 1986). Resemblance to bird droppings is seen in many butterfly larvae, e.g., in *Limenitis* and *Papilio* (Scott 1986), although to our knowledge the function has never been tested.

Late instar larvae of other *Polygonia* species (e.g., *P. interrogationis*, *P. comma*, *P. satyrus*, and *P. c-aureum*) and the related *Kaniska canace* are similarly colored in conspicuous white, black and orange, but with no continuous white areas (Scott 1986, Teshirogi 1990). This suggests the possibility that the coloration is aposematic in these species, and then perhaps in *P. c-album* and *P. faunus* as well. Here, we study the function of the coloration of late instar larvae of *P. c-album* using young chickens as models of bird predators. For comparison and as a control for effects of novel food, we also tested the chicks with third-instar larvae.

### MATERIALS AND METHODS

Females of *P. c-album* were captured near Akersberga, north of Stockholm, Sweden. Eggs were obtained in flight cages where the host plant *Urtica dioica* (Urticaceae) was present. Larvae were reared on this plant in the laboratory. Several asynchronous rearings

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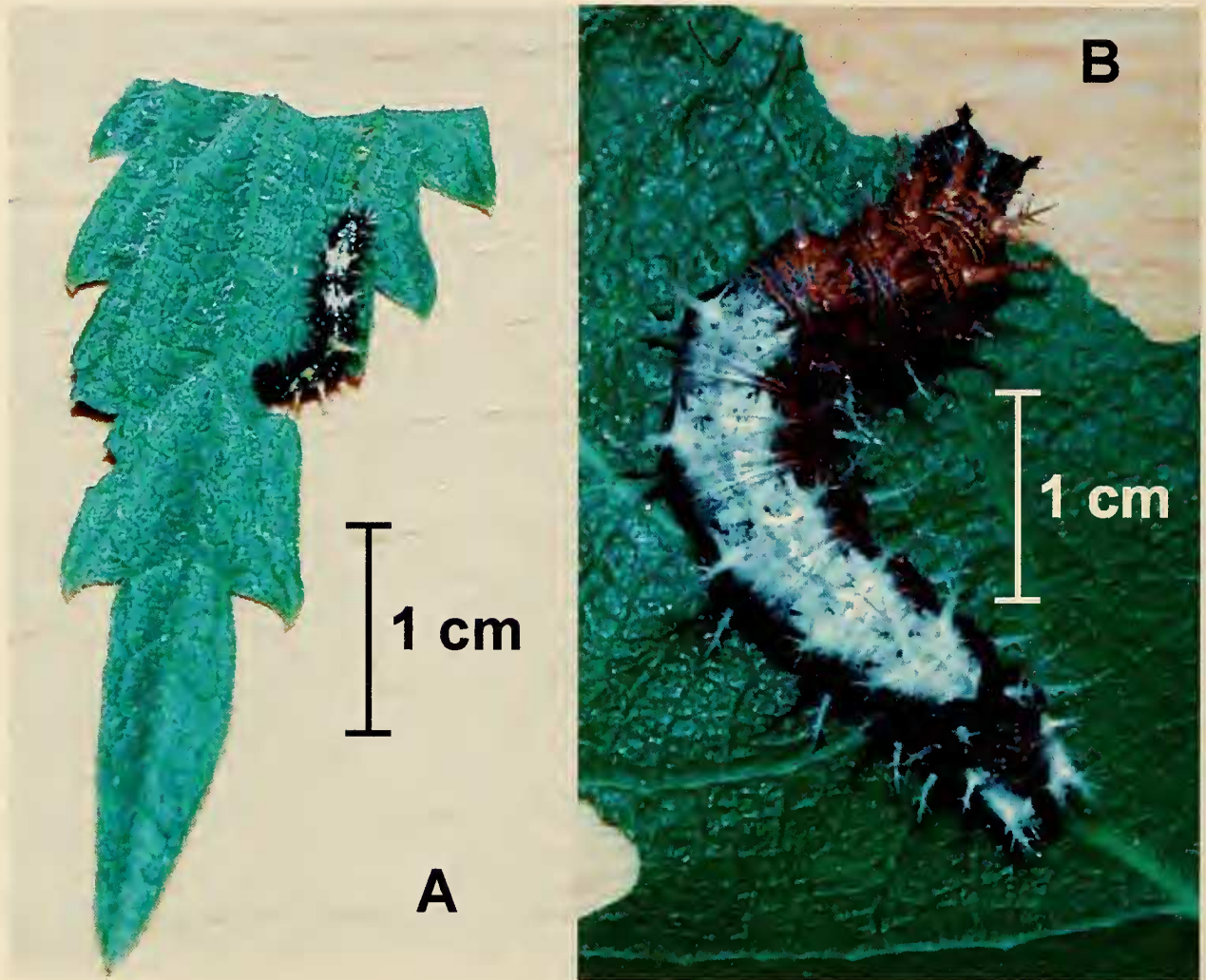


FIG. 1. Larvae of *Polygonia c-album* on leaves of *Urtica dioica*. A. Third-instar larva. B. Fifth-instar larva.

were made, so that third- and fifth-instar larvae were available for trial simultaneously.

**Third-instar larvae.** In this instar, the larvae lack large areas of any color except for the background color, black. Small spines are present, and towards the end of this instar the spines have a yellowish color on the foremost part of the body and a whitish color towards the rear (as seen in Fig. 1A). The impression to the human eye is not that of a striking coloration but rather a pattern that could function disruptively against a variegated background.

**Fifth-instar larvae.** In this instar, the foremost part of the body is colored orange, and the rear part is continuously colored white. The rest of the body is black with orange markings (Fig. 1B). Large, chitinous, branching spines are present on the back and sides of the body, and they are colored orange, white or black, according to position. Larvae are sev-

eral centimeters in length (Figs. 1A and B are approximately to scale).

**Chicks.** We used domestic chicks (*Gallus gallus domesticus*) as predators. The chicks had not eaten when they arrived from the hatchery at an age of less than 20 h. Batches of 30–40 chicks were housed in cages (100 cm × 55 cm × 20 cm) with wooden sides, steel-net floor, and a roof made partly of wood and partly of chicken wire. The cages were heated with 60 W carbon light bulbs and the floors were covered with sawdust. Chicks were fed chick starter crumbs and water, and at least from their second day on they were also fed live mealworms (*Tenebrio molitor*).

**Experiments.** The experiments took place in an arena of the same kind of cage in which chicks were housed. Part of the cage was screened off, leaving a testing floor of 30 cm × 55 cm. Experiments took place when the chicks were about three days old. The chicks

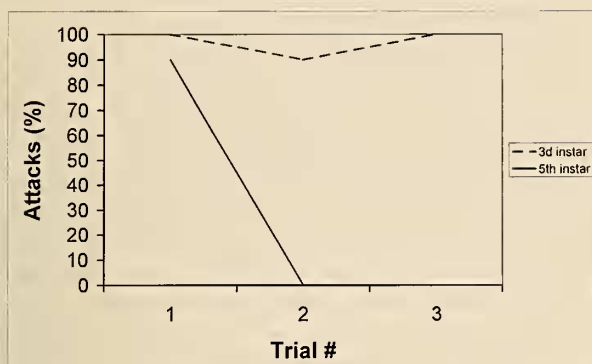


FIG. 2. Results from trials with domestic chicks presented with third-instar (dashed line) or fifth-instar (solid line) larvae of *Polygona c-album*. The same pair of chicks was tested on several occasions during one experimental day (subsequent trials from left to right). Both young and old larvae were attacked in initial trials, but fifth-instar larvae were avoided in the next trial.  $N = 10$  pairs of chicks in trial #1 and 2 for both larval instars,  $N = 7$  pairs of chicks in trial #3 with young larvae (see text).

were tested in pairs, because single chicks become distressed and do not feed normally (Gamberale & Tullberg 1996, and references therein). One of the chicks was fed as many mealworms as it would eat before a trial, which made it satiated and uninterested in feeding during the trial. This chick was used as a companion to the experimental chick. We used the same companion chick in all trials.

Prey was presented in a petri dish with transparent bottom, i.e., chicks saw the prey against a background of sawdust. Before each trial, the chicks were presented two mealworms in the petri dish. This was done to show the place where prey was displayed, and as a check before each trial that the chicks were in fact interested in insect prey. The chicks were then presented one mealworm and one larva of *P. c-album*. We collected data concerning chick attack behavior and the mortality of the attacked insects. Chicks were exposed to the prey throughout the trial and allowed to make as many attacks as they wished. A trial was ended when the *P. c-album* larva had been eaten, or after 60 seconds if the chick did not peck on the *P. c-album* larva at all. If it did, we waited another 60 seconds to see if the larva was eaten.

Twenty chicks (other than the companion chick) were used in total, originating from two separate batches. Half of them were tested with third-instar larvae and the other half with fifth-instar larvae, using chicks from both batches in both types of experiments. All chicks were tested twice with the same type of larva. In addition, to test for the presence of any aversion learning against third-instar larvae, seven chicks (of the same batch) were also tested with such larvae a third time. Finally, four of these chicks, that had eaten

or attacked third-instar larvae on all three occasions, were tested a fourth time. All trial runs were performed consecutively on the same day, within five hours. We used Fisher's exact test for statistical comparisons.

A follow-up experiment was performed next season, with new larval stock and a new batch of chicks, this time they were one week old. Five pairs of chicks were presented with a spineless fifth-instar larva together with a mealworm in a petri dish. The larvae had been killed by freezing and their spines had been cut off.

## RESULTS

**General observations.** The two mealworms presented at the start of each trial were always eaten rapidly, demonstrating that the chicks were very interested in insect prey. In all trials the chicks were initially more interested in attacking the mealworms than the comma larvae when subsequently given a choice, not surprisingly since this was a food type with which they had previous experience. The third-instar *P. c-album* larvae might also have been somewhat hard to see against the variegated background of sawdust. However, the experimental chicks were curious and in 19 out of 20 initial trials (both age classes combined) eventually pecked at the comma larvae.

**Third-instar larvae.** In the initial trials all 10 experimental chicks attacked the third-instar comma larvae (Fig. 2). In five of these cases the larva was also eaten (after a time span of, respectively, 19, 30, 35, 45, and 45 seconds). In the next trial the same chicks attacked in nine out of ten cases (decrease in attack frequency not statistically significant: Fisher's exact test), and ate the larva in two cases (both after 45 seconds). Both of these cases involved chicks which had also eaten the comma larva in the previous experiment.

As explained in Materials and Methods one group of chicks was tested a third time, with attacks by all seven experimental chicks (Fig. 2) and the larvae being eaten by the same two chicks (after 25 and 120 seconds). When the two chicks that had eaten the larva on all three occasions were tested anew they did so again, this time within 20 seconds after exposure. When two chicks that had attacked but not eaten (on all three occasions) were tested again they repeated this behavior. The last trials with this group took place 2 hours and 40 minutes after the start of the experiment.

In all cases when the larva was not eaten it was still alive after the experiment (despite having been pecked at), but on one occasion it was visibly damaged, and for this reason it was killed by us.

**Fifth-instar larvae.** The older larvae were never eaten by the chicks. In the initial trials attacks took

place in nine out of ten cases (Fig. 2). They were in the form of pecks, in three cases followed by the chicks lifting the larva and dropping it. In the next trial, which took place less than an hour after the first, the chicks behaved very differently. There were no attacks in the form of pecks (Fig. 2). The decrease in attack frequency between trials is statistically significant (Fisher's exact test: two-tailed  $p < 0.05$ , one-tailed  $p < 0.01$ ). In all cases except one ( $N = 10$ ) the experimental chick inspected the comma larvae closely, sometimes for periods up to 15 seconds. In two cases attacks were initiated but terminated before contact. Larvae were alive and apparently undamaged after all experiments, and they were returned to their host plants where they soon continued to feed.

**Fifth-instar larvae without spines.** In this experiment larvae from which the spines had been removed were presented to the chicks. The first pair of chicks did not eat the larva, but they showed no aversion and handled the larva throughout the experiment. This larva was presented also to the next pair of chicks, and it was then eaten within 10 seconds. The three additional pairs of chicks also ate the spineless larvae within seconds.

#### DISCUSSION

Five out of ten chicks in initial trials ate third-instar larvae, but only two chicks continued to eat them in subsequent trials. This suggests that they are not highly palatable to birds; possibly the small spines confer them a limited defense. On the other hand, chicks showed no other signs of beginning to avoid third-instar larvae after having experienced them. They were almost always attacked (even though the previous experience was less than an hour earlier), and two chicks did eat them in all four trials within a time span of less than three hours. Evidently no chemical defense effective against birds is present in the comma butterfly. This conclusion is also supported by the fact that the adults of the species are highly cryptic and also very palatable to great tits (*Parus major*) in laboratory experiments (SN & BST unpubl.). However, this evidence is not conclusive, since butterfly larvae can be unpalatable even when adults are palatable (Bowers & Farley 1990, Dyer & Bowers 1996).

The chicks showed no initial aversion to the older comma larvae, but very rapidly learned to avoid them. It seems highly improbable that a chemical defense should be present in old larvae but not in young larvae or adults. More probably, the strong and sharp spines are what defend older larvae, as demonstrated by the result of removing the spines. In any case, the rapid aversion learning suggests the possibility of an apose-

matic function of the striking coloration. In the absence of color manipulations we cannot, however, rule out the possibility that aversion was in fact to the sight of the spines themselves, independently of color. Interestingly enough, the spines are colored in such a way that might make them more conspicuous (Fig. 1B), so these two possibilities may be hard to separate. A dual function is also possible, so that the coloration indeed mimics bird droppings, as previously suggested (Thomas 1986) but has an aposematic function once the larva has been discovered. This would be useful if some predators are not deterred by the spines, or if larvae are often damaged by naive predators.

In this context it is, however, interesting to note that the fifth-instar larvae were not damaged by the attacks in the initial trials, when the chicks learned to avoid them. This is in line with previous results from chemically defended aposematic insects (Boyden 1976, Järvi et al. 1981, Wiklund & Järvi 1982, Wiklund & Sillén-Tullberg 1985) but has not often been investigated in mechanically defended prey (but see Carrick (1936) for results from the related butterfly *Aglais urticae*). The importance of such observations is that they demonstrate that individual selection for aposematic coloration is possible. In other words it is not necessary to invoke kin selection or other types of indirect selection, as would be the case if some individuals must be sacrificed before predators can learn aversion (Fisher 1930, Benson 1971). Individual selection is a more parsimonious explanation of aposematic coloration when direct benefits to the individual are present, because for indirect selection additional assumptions of starting conditions are necessary regarding prey family groupings and the movements and memory of predators. Indeed, even aposematic butterflies often have cryptic pupae, and this is the stage of the life cycle that is most likely to be killed by inspecting predators (Wiklund & Sillén-Tullberg 1985). Conversely, strong spines, which (as demonstrated here) should give direct benefits to the individual, are found very commonly in nymphalid larvae, often together with contrasting, bright colors or a jet-black color that should provide little crypsis against green leaves (e.g., in other Nymphalini and in the Kallimini and Argynniti). Spines are absent in some chemically defended groups such as the Danainae, with aposematic larvae, and also in groups such as the Satyrinae and Apaturinae, which have clearly cryptic larvae. In many cases, however, the patterns are far less clear and the function of the coloration uncertain (for instance, *Ladoga* larvae in the Limenitidini have spines but cryptic coloration; Teshirogi 1990).

The ontogenetic shift in defense tactics in *P. c-album*,

from cryptic coloration to aposematic coloration and mechanical defense (seen also in *A. urticae*; Carrick 1936), is understandable in terms of the general increase in size during larval growth, for several reasons. First, it may not be possible for a small larva to have spines large enough to deter birds and other vertebrate predators. Second, small larvae may be more vulnerable to attacks by vertebrate predators. If predators need to learn aversion, small larvae may not survive this process, and as a consequence it may not be a profitable strategy to advertise a degree of unpalatability (Gamberale & Tullberg 1996). Third, the increase in size during growth of butterfly larvae has been found to be coupled with a shift from predominantly predation by invertebrates to predominantly predation by vertebrates, such as birds (Kristensen 1994). Hence, the need for defense against vertebrate predators should be largest in the last larval instars. Fourth, it may be difficult to evolve a pattern that is effectively aposematic given a very small size of the colored areas. In tests with aposematic bugs (Lygaeidae), chicks more readily learned aversion towards to the larger late instars, even though the coloration is the same (Gamberale & Tullberg 1996).

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